DPYD variants of the HapB3 haplotype are not in complete linkage

Amy. J. Turner[1], Cyrine E. Haidar[2], Wenjian Yang[2], Erin C. Boone[3], Steven M. Offer[4], Philip E. Empey[5], Andrew Haddad[6], Gunter Scharer[1], Ulrich Broeckel[1] and Andrea Gaedigk[3,7]

RPRD Diagnostics LLC, Milwaukee WI; [2] Department of Pharmacy and Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN; [3] Division of Clinical Pharmacology, Toxicology & Therapeutic Innovation, Children's Mercy Research Institute (CMRI), Kansas City, MO; [4] Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN; [5] Department of Pharmacy and Therapeutics, University of Pittsburgh School of Pharmacy, Pittsburgh, PA; [6]Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA; [7] School of Medicine, University of Missouri-Kansas City, Kansas City, MO

Background

- ***DPYD***: encodes dihydropyrimidine dehydrogenase, the rate-limiting enzyme for the fluoropyrimidines 5-fluorouracil (5-FU) and capecitabine.[1]
- **Decreased DPYD activity:** Severe, often fatal toxicity due to reduced drug clearance (Intermediate and poor metabolizers).
- **The HapB3 decreased function haplotype:** c.1129–5923C>G (rs75017182, intron 10), which introduces a cryptic splice site and : c.1236G>A (p.Glu412=, rs56038477) a benign SNP in exon 11.
- **Haplotype LD:** Previous reports described these SNPs to be in perfect linkage disequilibrium (LD), and thus, the benign exon SNP is often being utilized as a tagSNP in lieu of detecting the function-altering intronic variant.

Methods

RPRD:

- Samples from St. Jude Children's Research hospital were genotyped on PharmacoScan™ array.

St Jude:

- Confirmatory Whole genome sequencing (WGS).

Children's Mercy (CMH):

- Next generation sequencing data (Center for Pediatric Medicine).
- Queried for samples that are homozygous for c.1236G>A and heterozygous for c.1129-5923C>G.

All of Us v7 cohort[3]:

- Samples (n=245,394) were analyzed to further substantiate incomplete SNP linkage.

Results

- Clinical genotyping of *DPYD* on PharmacoScan Array identified a patient who had c.1236G>A, but not c.1129–5923C>G suggesting that these two SNPs may not be in perfect LD as previously assumed.
- WGS performed by St. Jude confirmed the genotype results.
- CMH identified at Trio in which the mother was homozygous for c.1236G>A, and heterozygous for not c.1129–5923C>G **.** The father had no variants detected and the child was heterozygous for only c.1236G>A (**Figure 1**).
- Analysis of the All of Us v7 data identified additional individuals carrying alleles with only c.1236G>A.

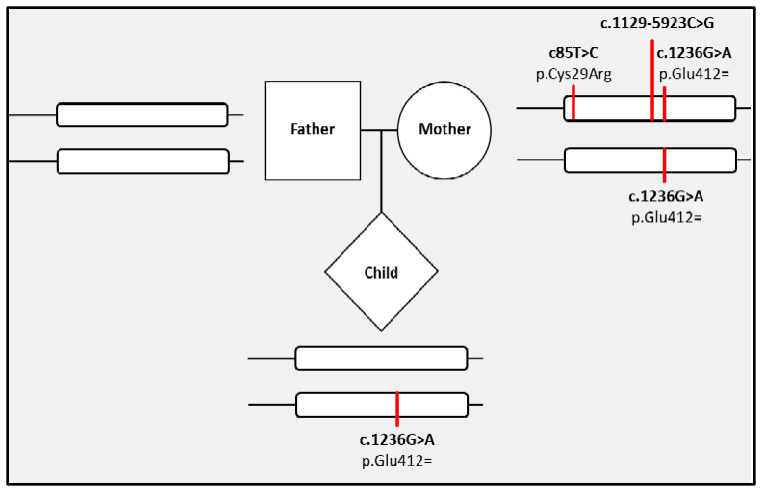

Figure 1: Pedigree of CMH trio demonstrating that c.1236G>A can occur by itself and is not in complete linkage with the causal variant, c.1129-5923C>G.

- **These findings directly impacted recommendations for *DPYD* variant testing and how the hapb3 allele is defined**.[2,5,6,7]

Conclusions

- Clinical genotyping using PharmacoScan, All of Us and CMH warehouse WGS data independently identified individuals who have only one of the two SNPs of the *DPYD* Hap3B haplotype unequivocally demonstrating that these variants are not in perfect LD.
- Consequently, testing only c.1236G>A may produce a false-positive result in some patients and lead to suboptimal dosing that may negatively impact a patient's therapy and prospect of survival. In other cases, this testing strategy may produce a false-negative result and put the patient at risk of toxicity when exposed to normal doses.
- Our data demonstrate that DPYD testing should include the functional SNP c.1129–5923C>G, and not the c.1236G>A tagSNP, to accurately predict DPYD activity.

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